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URINARY EXCRETION OF TERTIARY AMINO METHOXY METHYLENEDIOXY PROPIOPHENONES AS METABOLITES OF MYRISTICIN IN THE RAT AND GUINEA PIG

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SUMMARY

Two nitrogen-containing metabolites of myristicin (1-methoxy-2,3-methylenedioxy-5-allyl benzene) are excreted in the urine of rats and guinea pigs following oral or intraperitoneal administration. The major basic ninhydrin-positive urinary metabolite of myristicin in the rat is 3-piperidyl-1-(3'methoxy-4',5'-methylenedioxyphenyl)-1-propanone, while the major basic ninhydrin-positive urinary metabolite of the guinea pig is 3-pyrrolidinyl-1-(3'methoxy-4',5'-methylenedioxyphenyl)-1-propanone. In addition, the rat and guinea pig excrete trace quantities of the pyrrolidinyl ketone and piperidyl ketone, respectively. In contrast to the urinary basic metabolites of safrole, no detectable quantity of the *N,N* dimethylamino ketone was present in either rat or guinea pig urine after administration of myristicin; the guinea pig upon administration of safrole excreted only the substituted 3-*NN*-dimethyl amino propiophenone.

INTRODUCTION

Myristicin (1-methoxy-2,3-methylenedioxy-5-allyl benzene) has been isolated and identified in nutmeg[1], parsley[1], parsnip[2], carrots[3], bananas[4], black pepper[5], processed tobacco[6], and many other natural oils and flavoring agents with which individuals have daily contact.

Approximately 84% of the aromatic fraction of nutmeg[7] is composed of myristicin, elemicin, and safrole. In previously reported studies on nutmeg[7], myristicin has been recognized as a major component of the aromatic fraction. In general, myristicin[7] has been considered in part to be responsible for the psychopharmacological activity of the total nutmeg extract. Administration of purified myristicin[7,8] produces significant psychopharmacological responses but the effect is not as great as the total effect of nutmeg. In addition, myristicin also exhibits insecticidal and strong synergistic properties[2].

As indicated above, myristicin has some very unusual properties and a wide occurrence in the environment. The present investigations are a portion of a series of studies undertaken to firmly document the formation of nitrogen-containing metabolites and related derivatives from the propenyl benzene derivatives of essential oils.

An earlier report[10] from this laboratory described the production of basic ninhydrin-positive metabolites in the urine of rats and guinea pigs after administration of myristicin, safrole, isosafrole, asarone (*trans*) or β-asarone (*cis*). Complete structural identification of the safrole basic metabolites[11] indicated that the major basic rat metabolite of safrole was 3-piperidyl-1-(3′,4′-methylenedioxyphenyl)-1-propanone. The aminoketones derived from safrole[11] decomposed to 1-(3′,4′-methylenedioxyphenyl)-3-propen-1-one.

The present report describes the chemical structural verification of the ninhydrin-positive basic metabolites of myristicin. This report is further proof of the conversion of non-nitrogen containing propenyl benzene derivatives of essential oils to nitrogen-containing tertiary amino propiophenones.

MATERIALS AND METHOD

Both natural myristicin obtained from parsley seed oil[10] and synthetic myristicin[12] further purified by silicic acid chromatography[10,11] were administered (5–20 mg/kg) to rats and guinea pigs intraperitoneally. All of the myristicin used was 99% purity[10,11] or greater. The urine specimens were collected and stored as described earler[10,11].

Because of decomposition caused by prolonged exposure to alkaline conditions, the original extraction procedure[10] was modified slightly. The urine was first extracted at pH 1 with ether to remove the acidic *plus* neutral fractions and then the aqueous urine was adjusted to pH 13. One extraction of the pH 13 aqueous system with the required amount of ether produced the "basic -amine fraction" which contained the ninhydrin-positive metabolites.

Further investigation on the recovery of the basic metabolites with the synthetic 3-piperidyl-1-(3′,4′-methylenedioxyphenyl)-1-propane. HCl[11] under above modified extraction procedure indicated that 98% of the free tertiary aminoketone is removed by one extraction with ether at pH 13. By varying the pH and extracting under the above standardized condition only 4% and 85% of the theoretical amount of free base was extracted at pH 7.0 and pH 11.5, respectively.

All other analytical methods including thin-layer chromatography, infrared and mass spectroscopy were described for the safrole basic metabolites[11].

The synthetic 3-methoxy-4,5-methylenedioxyacetophenone (m.p. 81–83°; lit.[13] m.p. 82.5–83.5°) was prepared from the appropriate catechol[14] obtained from 5-iodoacetovanillone[15] which was prepared by iodination of acetovanillone (Aldrich Chemical Co., Milwaukee, Wisc.). All of the above intermediates leading to the formation of the final 3-methoxy-4,5-methylenedioxyacetophenone had the specified properties as described in the literature[13–15].

The three expected tertiary aminopropiophenones derived from myristicin were synthesized by the Mannich reaction[16] *via* the reaction of the above 3-methoxy-4,5-methylenedioxyacetophenone with paraformaldehyde and the appropriate secondary amine hydrochloride in ethanol. The 3-*N*,*N*-dimethylamino-1-(3′-methoxy-

4',5'-methylenedioxyphenyl)-1-propanone · HCl (yield 22%; m.p. 216–217°) and a very hydroscopic 3-pyrrolidinyl-1- (3'-methoxy-4',5'-methylenedioxyphenyl)-1-propanone · HCl (yield 13%; m.p. 185–187°) (free base m.p. 116–117°) were synthesized for the final structural verification of the myristicin metabolite. The Mannich salts were purified, and converted to the free base and to other appropriate derivatives as described earlier[11].

RESULTS AND DISCUSSION

In the case of nutmeg, myristicin has been considered in part to be responsible for a narcotic effect[7–9]. Administration of purified myristicin which is the major aromatic component of nutmeg produces significant psychopharmacological responses[7,8], but the effect produced by myristicin alone is not as great as that of total nutmeg. Earlier reports[7,8] indicate that myristicin produces a stimulant effect illustrated by a shortening of barbiturate sleeping time.

We have observed that upon intraperitoneal or oral administration of myristicin, safrole, isosafrole, asarone (*trans*), β asarone (*cis*), elemicin, eugenol and other propenyl benzene derivatives, rats and guinea pigs initially are highly active and excited. During the initial 15–30 min after administration of the propenyl benzene derivatives the animals are hyperactive. After about 30 min, the animal in the highly exicted state becomes very sedated, immobile and non-responsive to sound and motion for periods up to 2 h. All of the above described propenyl benzene derivatives seem to produce a stimulant and then a tranquilizing effect. More detailed studies with these propenyl benzene derivatives, their proven nitrogen-containing tertiary amino propiophenone metabolites and other precursor metabolites are in progress to more fully elaborate their psychoactive properties.

Metabolite I

The predicted major metabolite[11] of myristicin in the guinea pig (Metabolite I) would be 3-*N*,*N*-dimethylamino-1-(3'methoxy-4',5'-methylenedioxyphenyl)-1-propanone with a parent mass *m/e* 251 and a base peak of 58. On the contrary, no detectable *N*,*N*-dimethylamino-substituted propiophenone was found in guinea pig and rat urine after administration of myristicin.

Metabolite II

The major basic myristicin metabolite of the rat has a relative R_F in the methannol system[11] of 2.1 and is chromatropic positive[11]. The myristicin Metabolite II reacts with ninhydrin upon heating to yield a dark purple material. Fig. 1 illustrates the presence of the conjugated carbonyl absorption at 1670 cm^{-1}. Upon reduction of rat Metabolite II with sodium borohydride in ethanol[11], the carbonyl group is reduced to an alcohol with absorption at 3400–3300 cm^{-1}. As seen with the tertiary aminoketone metabolites of safrole[11], the reduced tertiary aminopropiophenones can be acetylated with acetic anhydride in pyridine to yield the monoacetyl derivative with a 1730 cm^{-1}; carbonyl absorption and no hydroxy absorption.

The myristicin rat Metabolite II has a parent mass of 291 with a base peak of *m/e* 98, less abundant 206, 179, 163, 151, and 133 fragments, and no significant fragments in the region between *m/e* 206 and 291. Upon reduction with sodium boro-

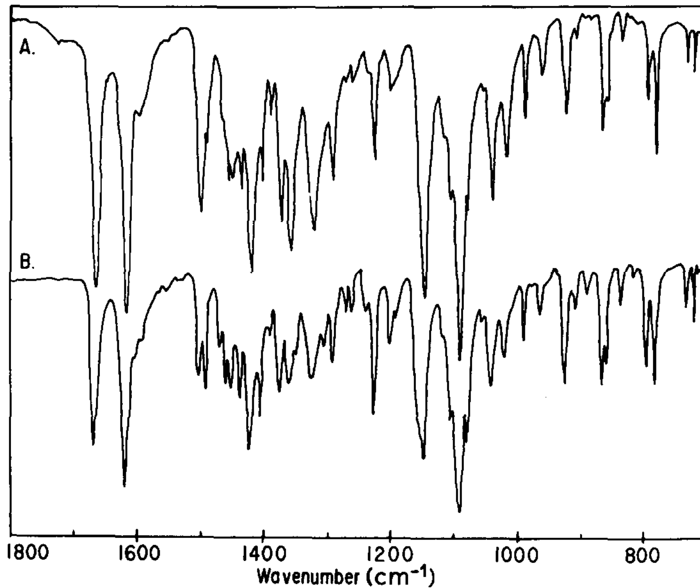

Fig. 1. Infrared spectra. (A) Rat Metabolite II of myristicin; (B) synthetic 3-piperidyl-1-(3'-methoxy-4'-5'-methylenedioxyphenyl)-1-propanone.

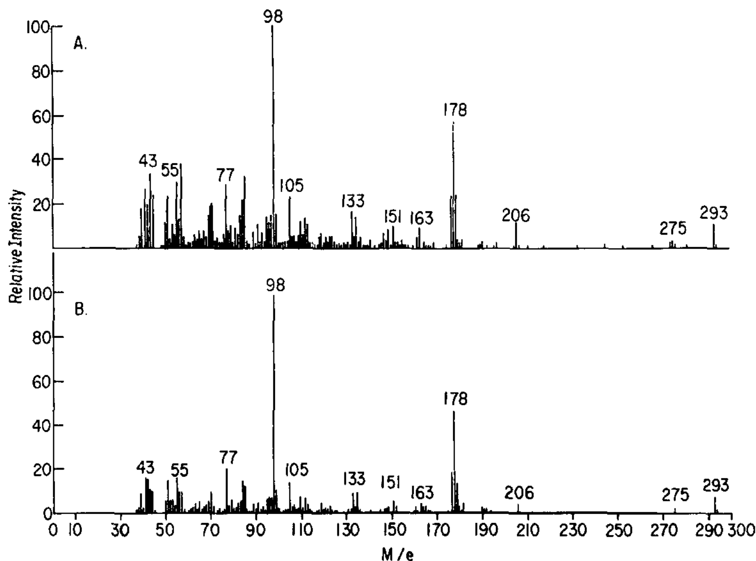

Fig. 2. Mass spectra. (A) Sodium borohydride-reduced rat Metabolite II of myristicin; (b) sodium borohydride-reduced synthetic 3-piperidyl-1-(3'-methoxy-4',5'-methylenedioxyphenyl)-1-propanone.

hydride, this derivative has a parent mass of 293 with a significant (P-18) fragment of 275, including 206 and 178 fragments. The base peak of the reduced Metabolite II remains *m/e* 98 (Fig. 2). Acetylation of the reduced Metabolite II (*m/e* 293) produced the monoacetylated derivative with a parent mass of 335 and a very prominent 292 fragment. The infrared and mass spectral data described above in comparison with

the organic sysnthesized standard derivatives, confirm the fact that the major basic urinary myristicin metabolite of the rat (Metabolite II) is 3-piperidyl-1-(3'-methoxy-4',5'-methylenedioxyphenyl)-1-propanone.

Metabolite III

The guinea pig excretes trace amounts of Metabolite II—3-piperidyl-1-(3'-methoxy-4',5'-methylenedioxyphenyl)-1-propanone. In the earlier report describing the metabolites of safrole[11], we did not detect any significant quantity of the substituted piperidyl propiophenone in guinea pig urine. In the case of the myristicin metabolites, the guinea pig excretes trace quantitites of the piperidyl propiophenone instead of the expected *N,N*-dimethylamino propiophenone. On the contrary, the major basic ninhydrin-positive metabolite of myristicin in the guinea pig has a relative R_F in the methanol system[11] of 0.9 and is chromatropic positive[11]. This metabolite upon spraying with ninhydrin and then heating yields a bright yellow material.

Infrared spectra of the myristicin Metabolite III of the guinea pig indicate the conjugated carbonyl absorption at 1670 cm^{-1} as described for the above Metabolite II and also that of safrole.

The major guinea pig metabolite of myristicin has a parent mass of 277 with less abundant 206, 179, 151, and 133 fragments. The base peak of Metabolite III is *m/e* 84 (Fig. 3). Reduction of guinea pig Metabolite III with sodium borohydride produced the alcohol with a mass of 279 and a (P-18) fragment of mass 261 with abundant 178 fragment. The base peak of the alcohol formed from Metabolite III remains *m/e* 84.

After comparison of myristicin Metabolite III derivatives of the guinea pig with the specific standard synthetic derivatives it is concluded that the major myristicin metabolite of the guinea pig is 3-pyrrolidinyl-1-(3'-methoxy-4',5'-methylenedioxyphenyl)-1-propanone. The trace metabolite of myristicin in the rat (III)

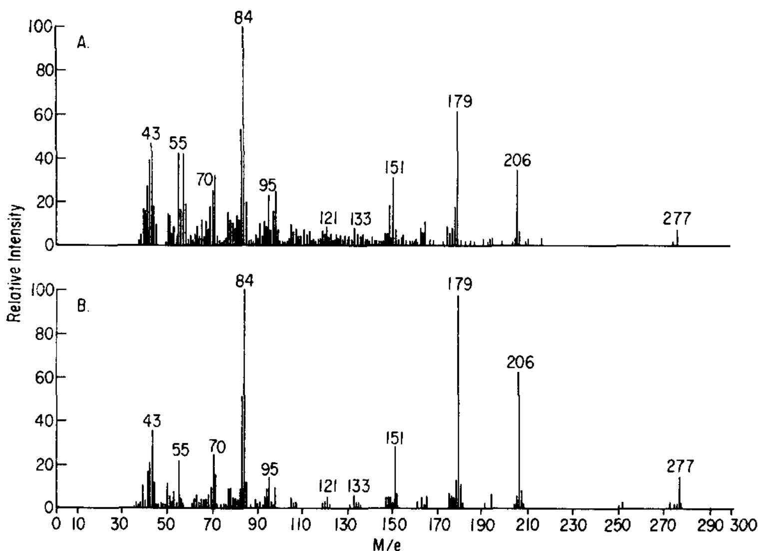

Fig. 3. Mass spectra. (a) Guinea pig Metabolite III of myristicin; (b) synthetic 3-pyrrolidinyl-1-(3'-methoxy-4',5'-methylenedioxyphenyl)-1-propanone.

was shown by the above criteria to be 3-pyrrolidinyl-1-(3′-methoxy-4′,5′-methylenedioxyphenyl)-1-propanone.

As described for the safrole metabolites[11], these substituted tertiary aminopropiophenones decompose on exposure to heat, alkaline conditions, silica gel, and air with formation of the substituted allyl ketone *plus* the secondary amine, piperidine or pyrrolidine. The piperidine or pyrrolidine formed in the heating process[11] reacts with ninhydrin to yield the highly colored derivative.

The present study as well as the previous study with safrole[11] illustrates that non-nitrogen containing allyl benzene derivatives are converted biologically *in vivo* in the rat and guinea pig to nitrogen-containing tertiary amino substituted propiophenones. The production of these basic ninhydrin positive metabolites of myristicin, safrole, and other propenyl benzene derivatives requires the presence of double bond in the propyl side chain.[10] Figure 4 outlines a possible mechanism for the biosynthesis of the tertiary amino substituted propiophenone from allyl benzene derivatives. This mechanism suggests that the allyl benzene derivative undergoes a biological allylic oxidation first to form the allylic ketone. The allylic ketone could then condense with a secondary amine, piperidine, pyrrolidine or dimethylamine in the presence of the appropriate enzyme systems to yield the final excreted tertiary amino ketone.

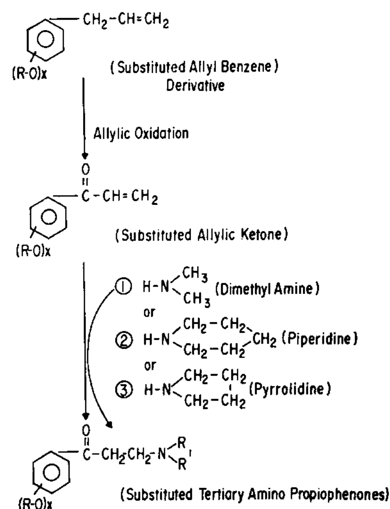

Fig. 4. Proposed pathway for biosynthesis of tertiary amino propiophenones from non-nitrogen containing components of essential oils.

All of the tertiary amino propiophenones described presently and for safrole[11] may very likely be the active metabolites which elicit the psychotropic activity of the propenyl benzene derivatives of essential oils. We also find that elemicin, eugenol and other substituted allyl benzene derivatives are metabolized to the same type of nitrogen-containing metabolites. The additive family of tertiary amino substituted propiophenones derived from all the allyl benzene derivatives of nutmeg may be the agents responsible for the total psychotropic properties of nutmeg.

Only after diverse areas of investigations involving the tertiary amino propiophenones, their precursor metabolites and propenyl benzene derivatives have been

completed, will we be able to more fully understand the physicological, psychotropic and pathological actions on the individual through his exposure to these environmental agents.

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